Non-Destructive *Ex Vivo* And *In Vivo* Metabolic Profiling Using High Resolution ^{1}H NMR

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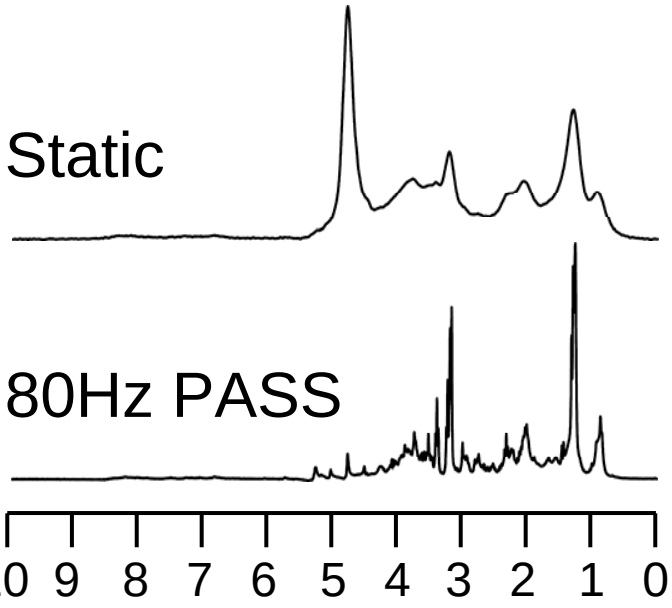
Research Objectives

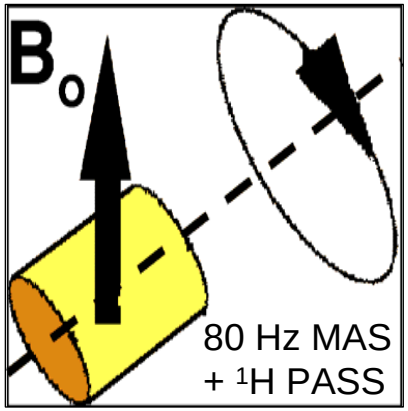
- To develop high resolution ^{1}H NMR capabilities that are suitable for non-destructive metabolic profiling *ex vivo* on excised tissues and key body fluids, and *in vivo* in live small animals.

- To identify potential metabolic markers in pulmonary inflammation and fibrosis caused by inhaled particulates. We will report results from preliminary studies on silicosis induced by crystalline silica dusts via intratracheal instillation.

Non-destructive slow-MAS ^{1}H NMR

metabolic profiling of intact excised C57BL/6 mouse lung

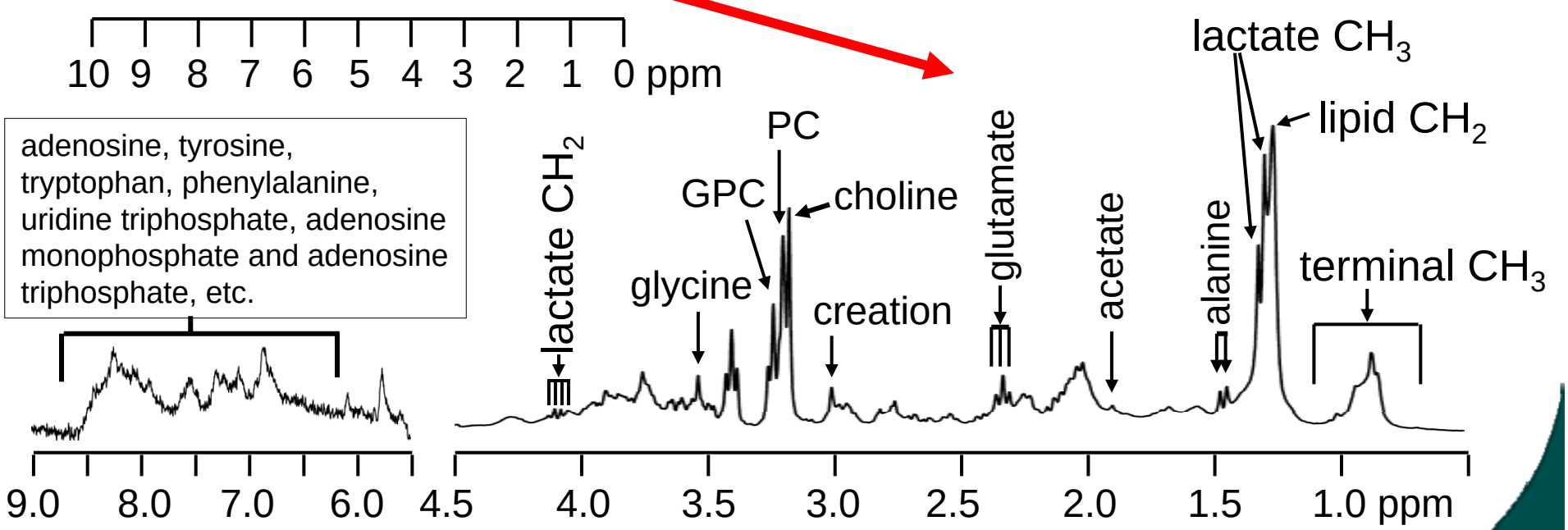




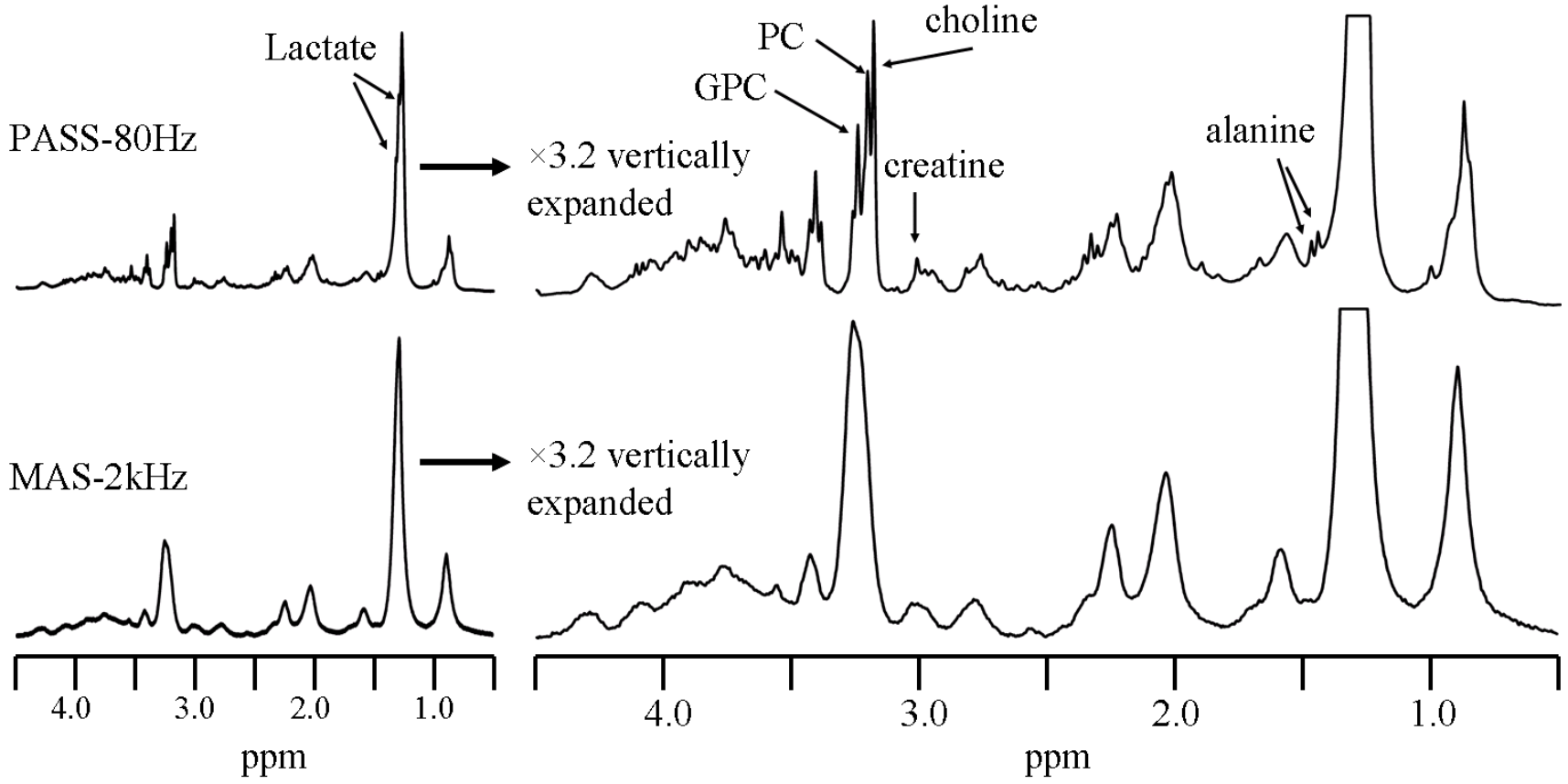
1. Wind, Hu and Rommereim, Magn Reson Med 46: 213-218 (2001).

2. Antzutkin, Shekar and Levitt. J Magn Reson A115: 7-19(1995)

adenosine, tyrosine, tryptophan, phenylalanine, uridine triphosphate, adenosine monophosphate and adenosine triphosphate, etc.

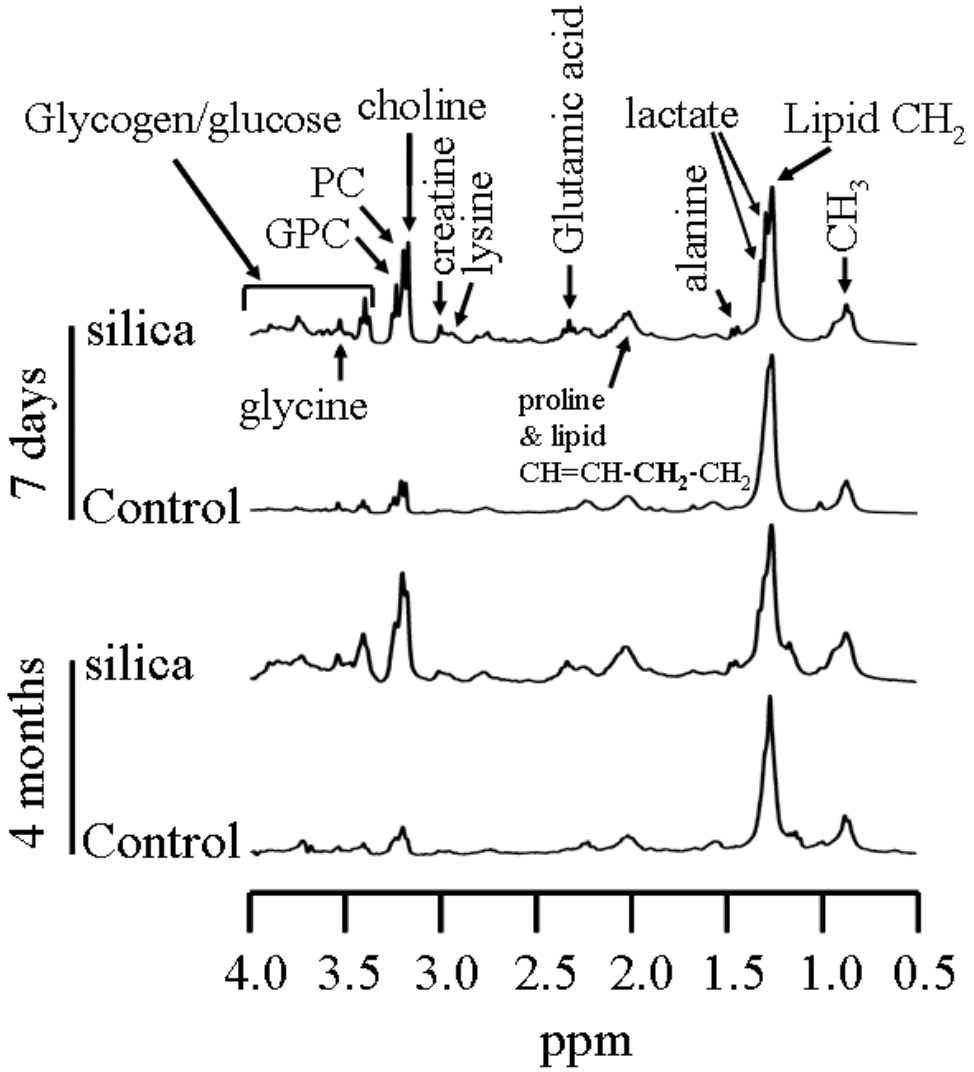


metabolic profiling of intact excised 89mg C57BL/6 mouse lung ^{1}H PASS (80Hz) versus fast-MAS (2kHz)



- Using the same experimental setup, we have found that slow-MAS 80Hz ^{1}H PASS always offers better spectral resolution than fast-MAS (2kHz).

^{1}H PASS Spectra of Excised Left Lungs of C57BL6 Mice 7 Days and 4 Months Post Silica Treatment



Results

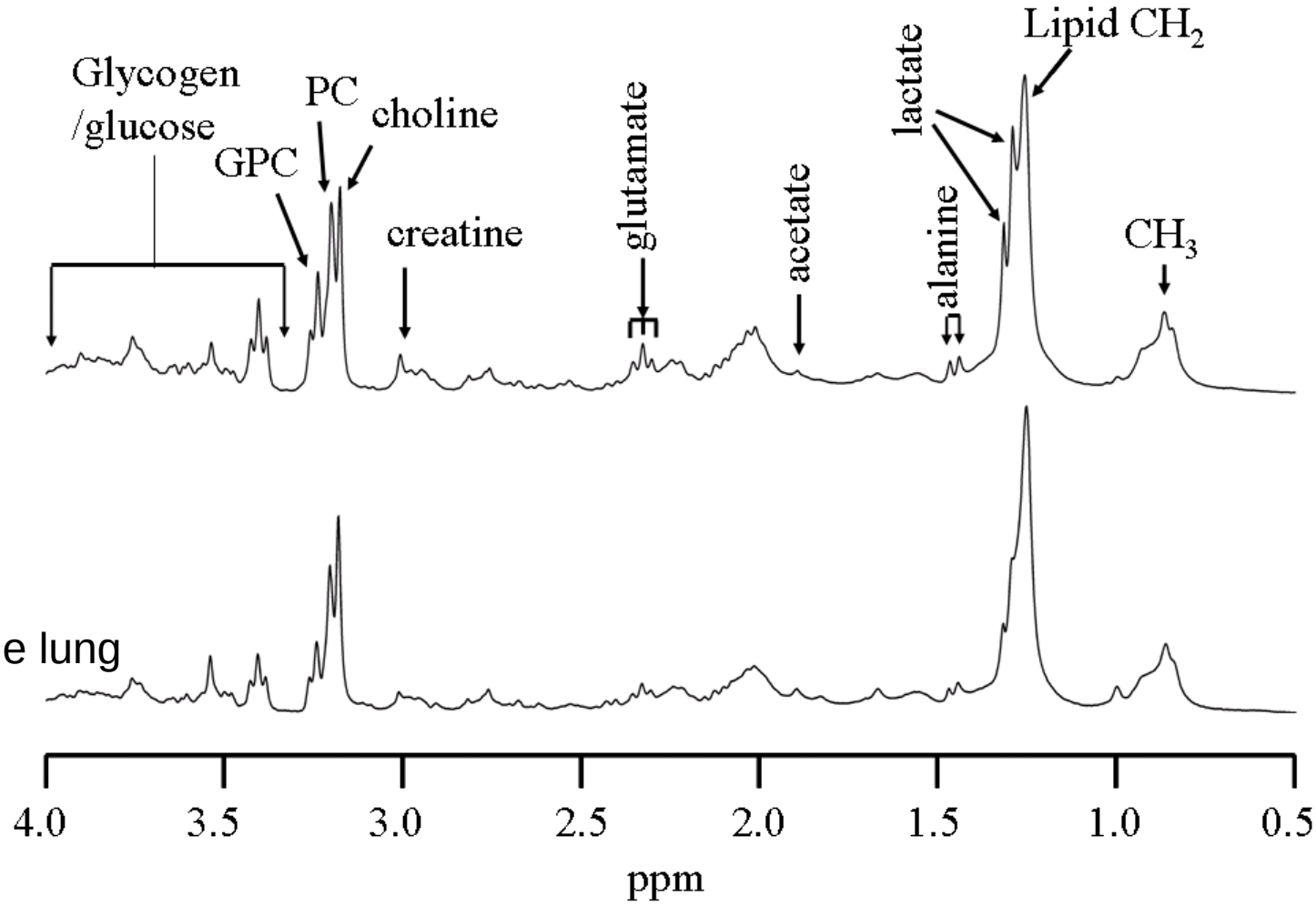
- The levels of choline, Phosphocholine (PC) and glycerophosphochiline (GPC) are significantly increased in silica (5mg) treated mice than control (saline treated).

- Lactate along with collagen metabolites: glycine, lysine, glutamate and proline are also significantly increased in silica treated mice.

^{1}H PASS Spectra of Excised Left Lungs of C57BL6 Mice 7 Days Post 5mg Silica Treatment: Spatial Heterogeneity of Metabolites

80mg from the middle section of the left lung

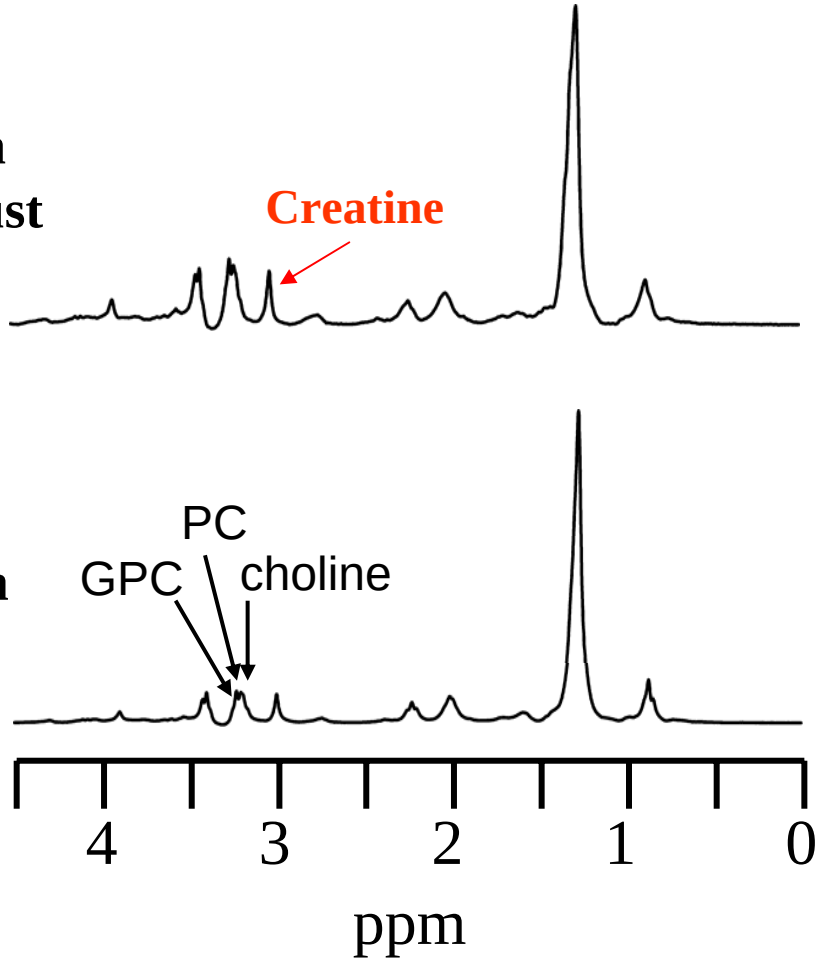
80mg from the lower lobe of the lung



^{1}H PASS Spectra of Intact Excised Heart of Mice 7 Days Post Silica Treatment

Instilled with 5mg silica dust

Instilled with saline



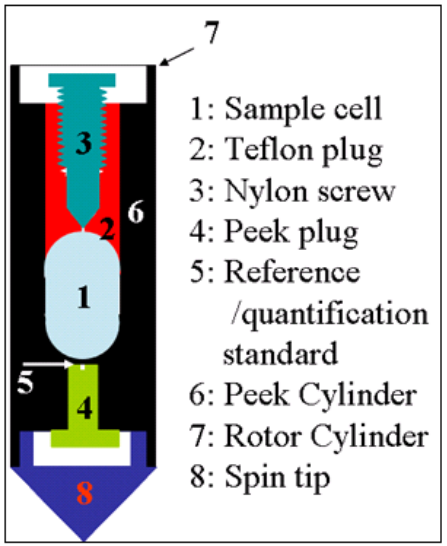
Results

- The levels of creatine, PC and GPC are elevated by a factor of more than two in silica (5mg) exposed mouse, compared with those from the saline control mice.

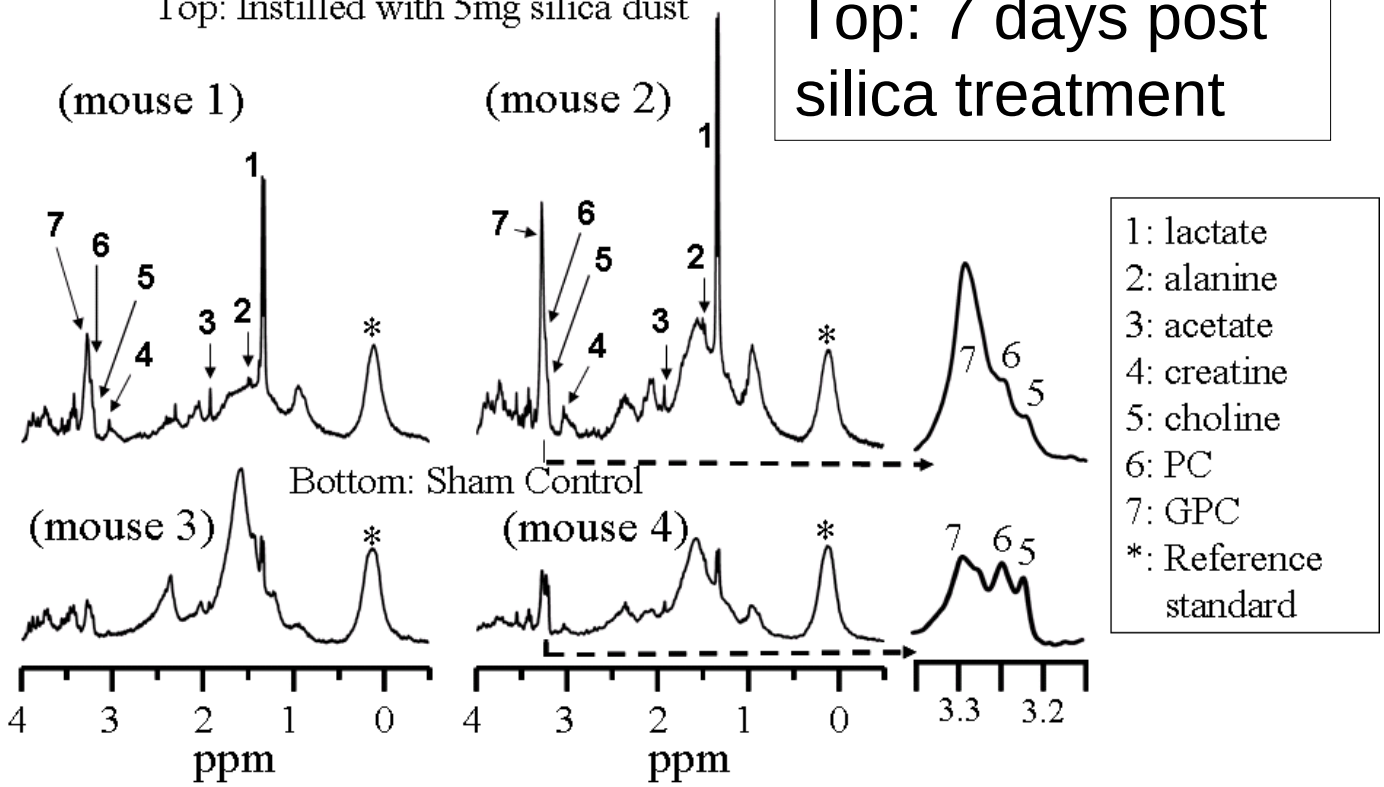
- This finding reveals that the inflammation that occurs in the lung also influences vital distal organs including the heart.

Metabolic Profiling of Bronchoalveolar Lavage Fluid (BALF) Using A Special **Liquid Tight MAS Cell** (80-120μl)

Liquid-tight MAS Cell
Capable of sample
spinning up to 3KHz



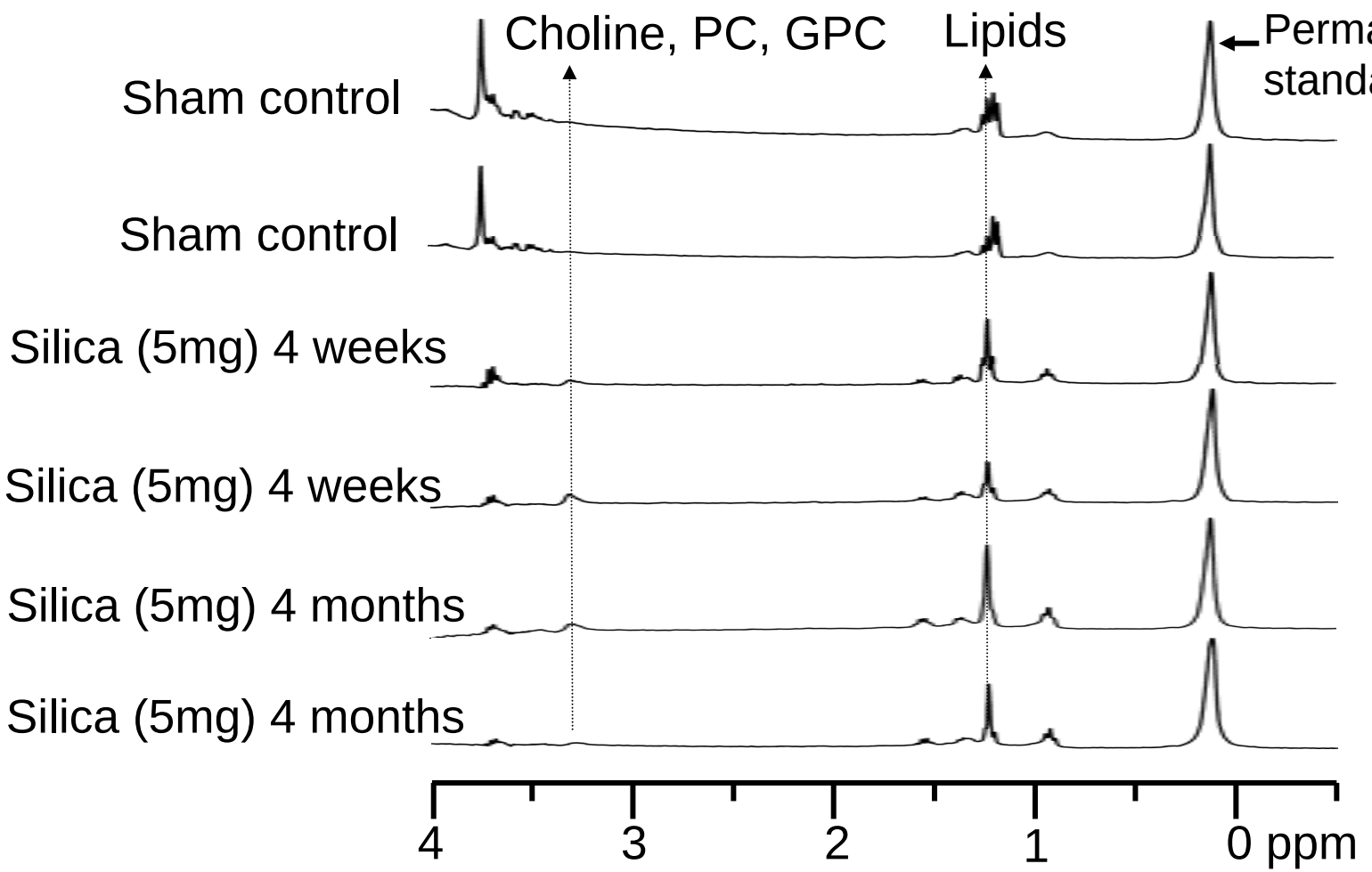
Top: Instilled with 5mg silica dust



Top: 7 days post silica treatment

Results: (1) Metabolites with concentration as low as **50μM** can be detected at 7T (300MHz); (2) Lactate, acetate and GPC are significantly elevated in BALF from mice treated with 5mg silica.

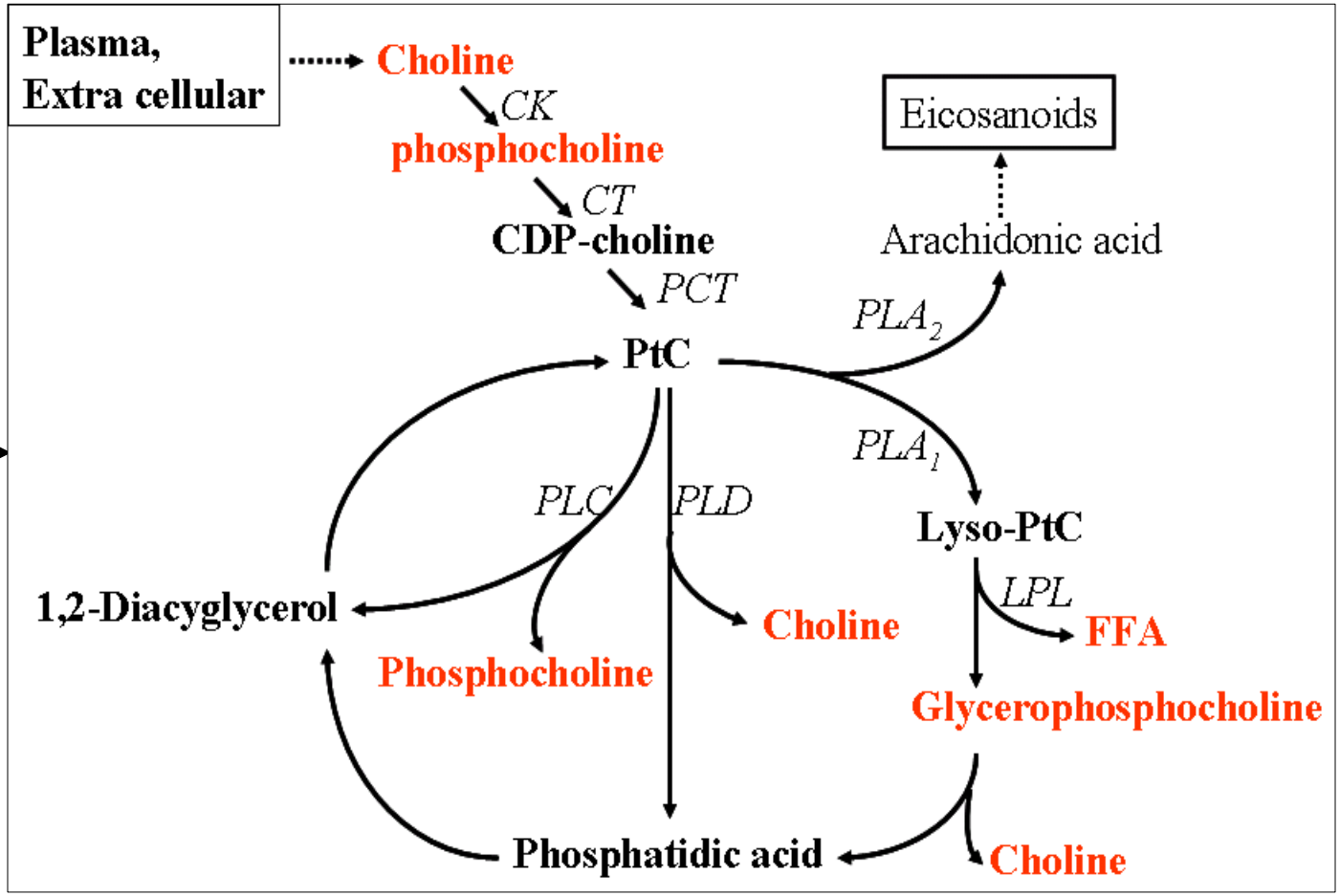
^{1}H fast-MAS (2kHz) NMR Metabolic Profiling of BALF from C57BL6 Mice Treated with Silica at Long Post Exposure Times



Results: Lipids are significantly elevated in BALF from silica exposed mice at long post exposure times.

Our Results Indicate that Membrane Choline Phospholipid Metabolism (MCPM) is one of the dysregulated pathways in pulmonary inflammation and fibrosis

MCPM →



Previously malignant transformation in human breast cancer cells has been reported to alter MCPM (Aboagye and Bhujwalla, Cancer Research 59, 80 (1999))

Other pathways that are alternated in silicosis include:

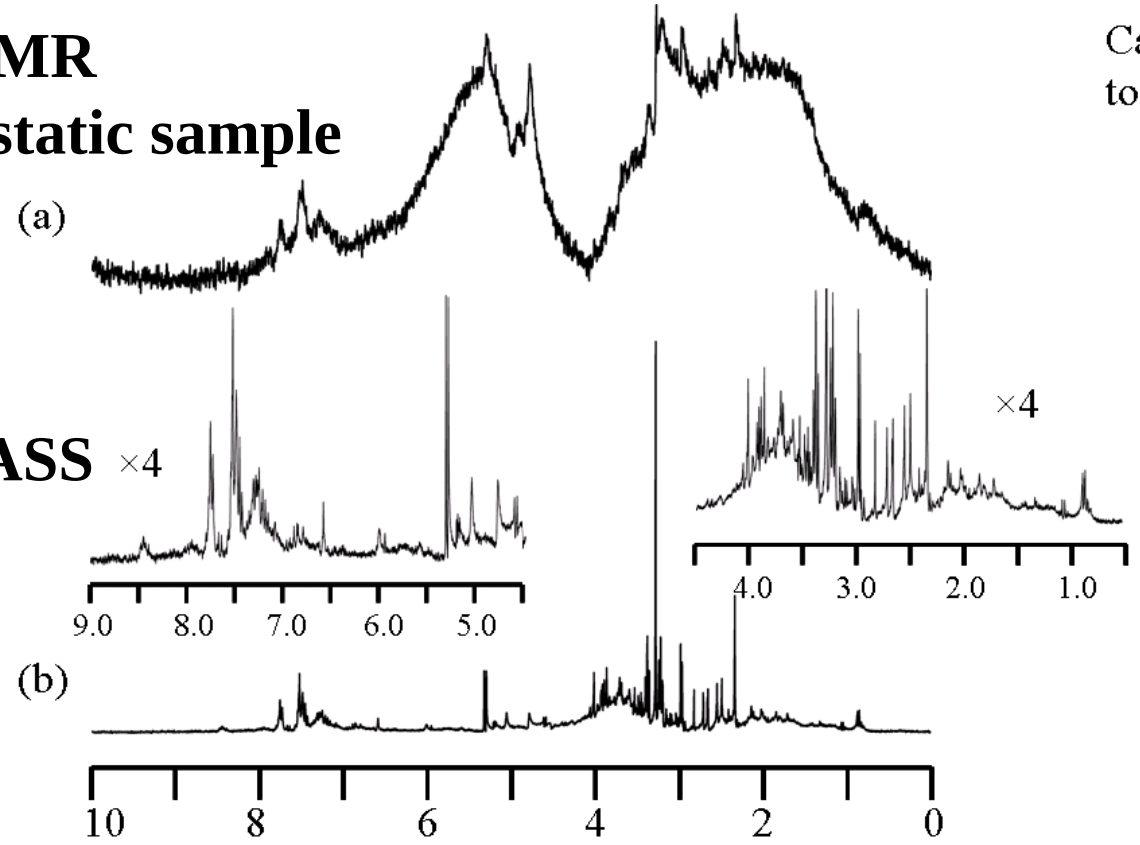
- **Lactate/acetate/glucose/glycogen pathway**

- **Collagen pathway *via* collagen metabolites: glycine, lysine, glutamate and proline**

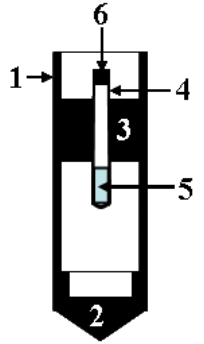
Slow-MAS Capillary Sample ^{1}H NMR for None-Invasive/Minimal Invasive Metabolic Profiling on Key Body Fluids with Limited Sample Supply

^{1}H NMR on a static sample

80Hz ^{1}H PASS



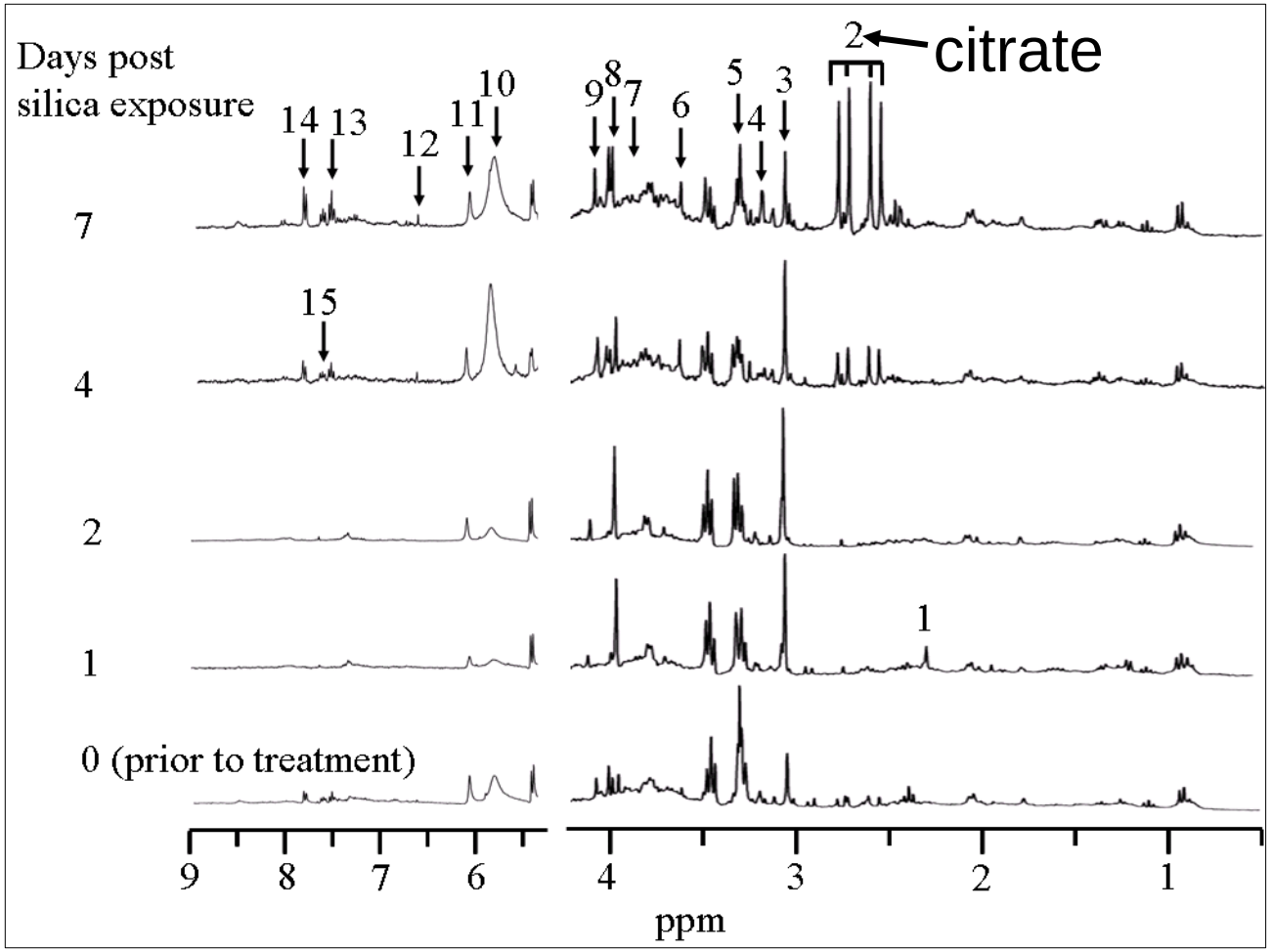
Capillary sample cell used to acquire the left spectra



1: Rotor cylinder
2: Flat drive tip
3: Teflon spacer for holding the capillary tube
4: Capillary sample tube
5: Sample
6: Cap of the sample tube.

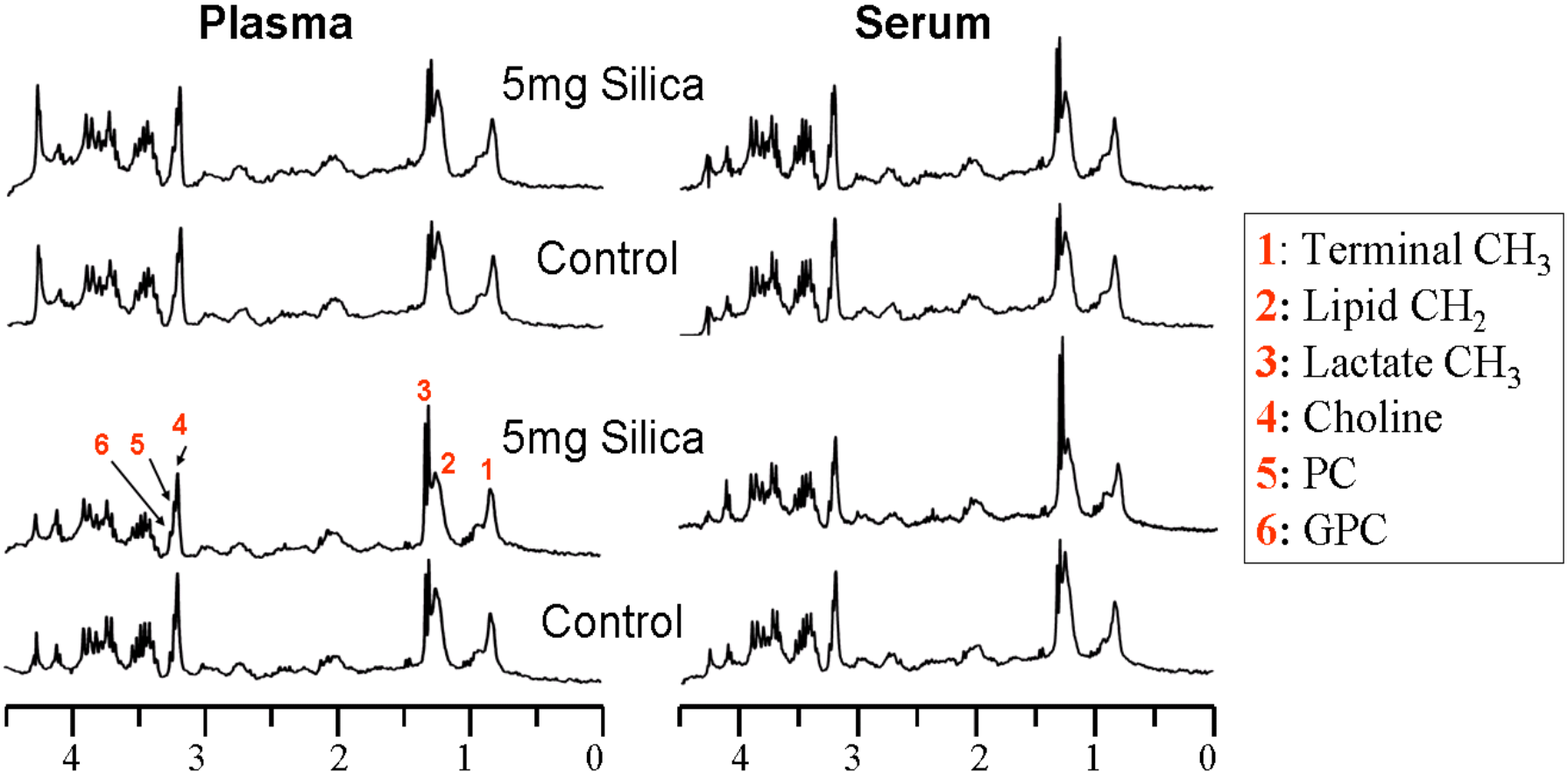
A large range of metabolites can be detected using our slow-MAS capillary NMR method on intact **mouse urine** with volume of only **2.5μl**. **Micro RF coil can be used to enhance the S/N.**

Slow-MAS Capillary Sample ^{1}H NMR of Urine of Silica (5mg) Exposed C57BL6 Mouse as A Function of Time Post Treatment



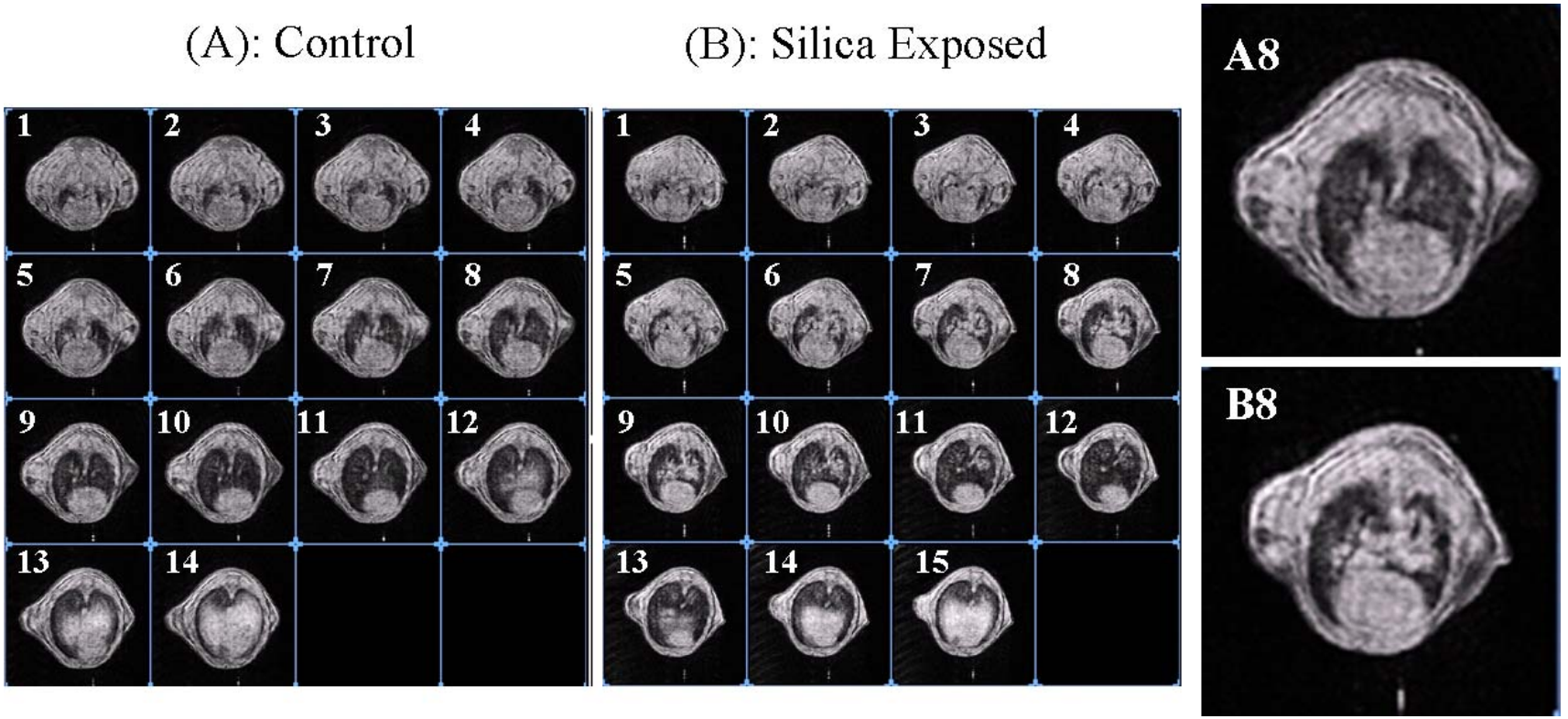
Urine samples with volumes of 2.5 to 8μl or more were collected at 9:00am ± 30 minutes at the targeted days to minimize the effects from normal biological variations such as the differences in excise, etc.

Slow-MAS ^{1}H NMR of ~5μl Plasma from Silica (5mg) And Sham Control Exposed C57BL6 Mice 7 Days Post Treatment



Results: (i) Similar information can be obtained from either plasma or serum; (ii) Lactate is elevated in silica exposed mice, consistent with BALF studies.

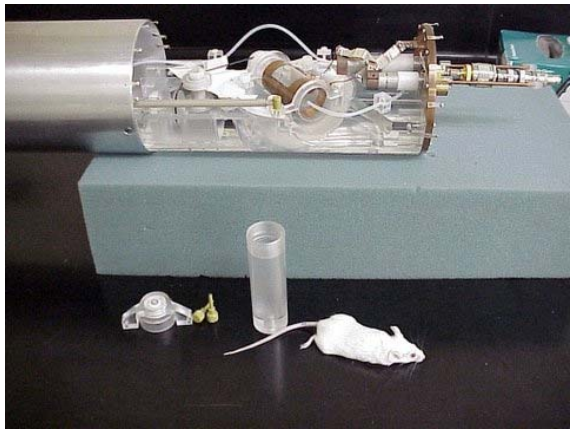
In vivo MRI for monitoring pulmonary inflammation in a live mouse



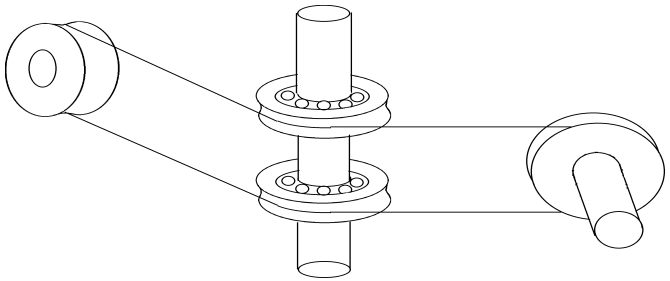
Imaging slices of 80MHz 3D ^{1}H MRI of BALBc mice. (A) The control mouse that was instilled with saline; (B) Mouse was instilled with 5mgsilica dust. Both (A) and (B) were obtained 14 days after the intratracheal instillation. A8 and B8 are expansions of the corresponding images in (A) and (B).

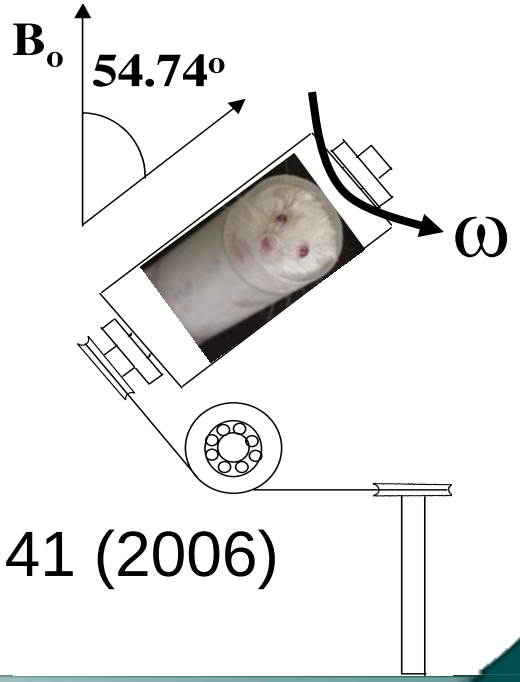
Ultra-Slow-MAS for *In Vivo* Whole Body and Localized High Resolution ^{1}H NMR Metabolic Profiling

(A) The probe



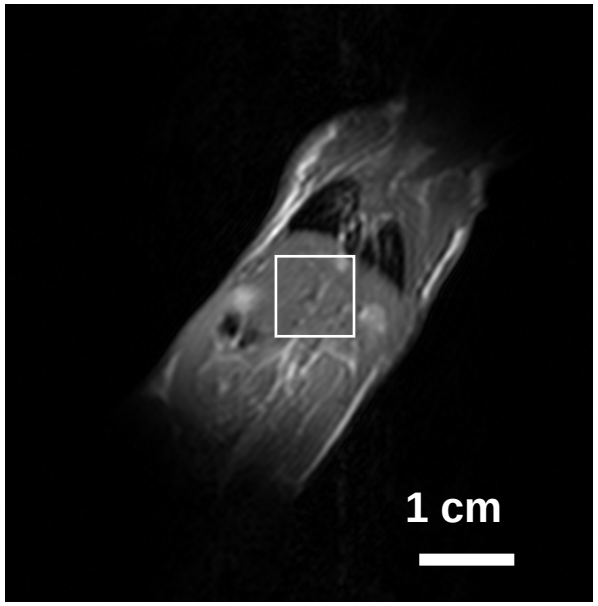
(B) The Rotation Mechanism



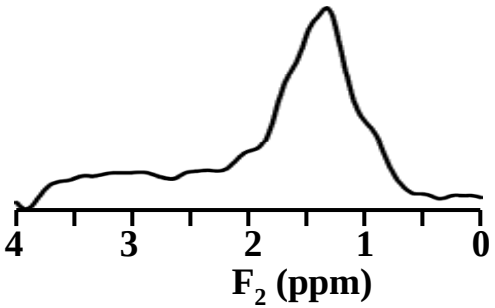


Wind, Hu and Majors. Magn Reson Med 55: 41 (2006)

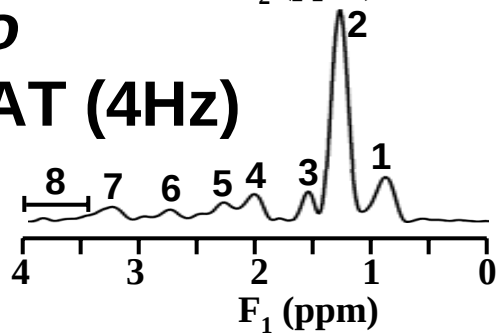
In vivo localized Ultra-Slow-MAS NMR Spectroscopy in Live Mouse (LOCMAT)



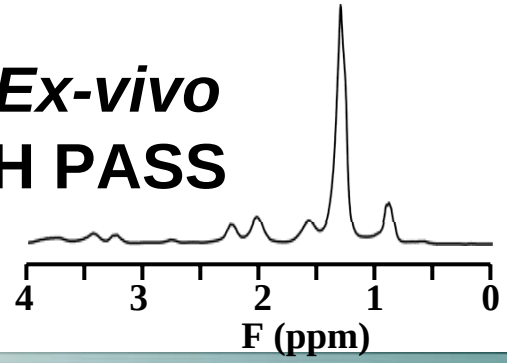
From traditional *In Vivo* NMR (static sample)



***In Vivo* LOCMAT (4Hz)**



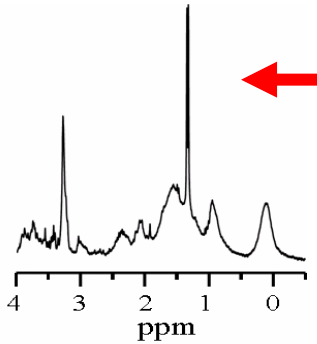
From *Ex-vivo* 40Hz ^{1}H PASS



Summary of Special ^{1}H NMR Metabolomics at PNNL

Intratracheal Instillation of silica dust

2-3kHz MAS (liquid-tight sample cell)

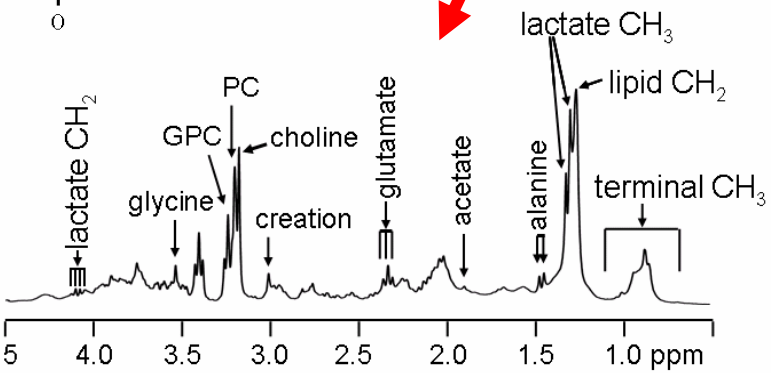


BALF

lung

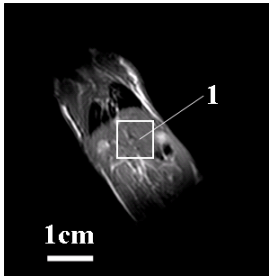
Invasive

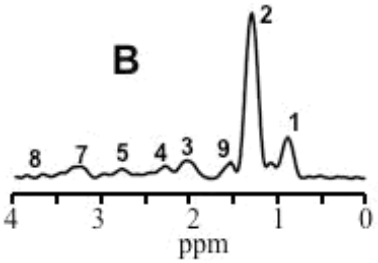
80Hz-PASS



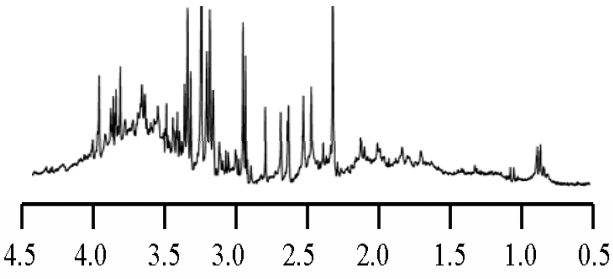
None/minimal-invasive Methods

***In vivo* localized slow-MAS NMR Spectroscopy (4Hz)**

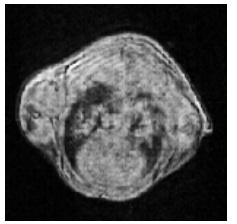




Urine, blood NMR Metabolic Profiling (2.5µl), 80Hz-PASS



***In Vivo* MRI (stationary)**



Acknowledgement

PNNL EBI-LDRD
PNNL IR&D
DOE-BER
NIH/NIBIB

Thank you!

Questions and Collaborations are very welcome!

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